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Membrane Traffic: GGAs Sort Ubiquitin

Dispatch

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Membrane proteins that are tagged with ubiquitin are diverted from the secretory pathway into lysosomes. New work shows that it is the GGA proteins that initiate sorting in the Golgi, and suggests that similar principles apply to multiple sorting steps.

Ubiquitin is a 76-residue polypeptide, the carboxyl terminus of which can be linked by a peptide bond to the amino groups on lysine side-chains, including those on other ubiquitin molecules. For cytoplasmic proteins, the consequences of polyubiquitylation are well-known: the modified proteins are degraded by the proteasome. A similar fate is suffered by malfolded or abnormal proteins in the endoplasmic reticulum (ER) — they are polyubiquitylated, extracted from the membrane and degraded, an important form of quality control [1]. For membrane proteins that escape the ER, an alternative ubiquitin-dependent route to destruction exists. Attachment of even a single ubiquitin can be sufficient for targeting from the Golgi [2], or from the cell surface [3], to endosomes, from there into the internal membranes of multivesicular bodies and finally to lysosomes or, in yeast, the vacuole [3,4].

These pathways are used for a variety of purposes: the downregulation of signalling receptors and ion channels; the removal of abnormal proteins; the regulation of amino acid and other transporters according to growth conditions; and the delivery of some vacuolar enzymes. Several proteins have been identified that recognise ubiquitin and control protein sorting at the plasma membrane and endosomes, but until recently sorting at the Golgi was unexplained. It is now clear that the GGA — Golgi-localized, gamma-ear-containing, Arf-binding — proteins can be added to the list of ubiquitin-binding proteins, and this goes a long way towards completing and unifying the sorting story [5–7].

GGA proteins are monomeric adaptors that are recruited to the trans-Golgi network by the Arf1 GTPase (reviewed in [8]). They consist of four distinct segments: a VHS domain that binds an acidic di-leucine sorting signal found in the mannose-6-phosphate receptor; a GAT domain which binds Arf:GTP; a hinge region which recruits clathrin; and a GAE domain which exhibits sequence similarity to the ear region of gamma adaptin and recruits a number of accessory proteins (Figure 1). GGAs thus have the ability to sequester endosome-bound receptors into clathrin-coated vesicles. What is now apparent is that the GAT domain has two quite distinct binding sites, one for Arf:GTP and another for ubiquitin [6,7]. Both ligands can bind simultaneously, and moreover Arf binding activates ubiquitin binding, which in the full-length GGA proteins is very weak [7]. This provides a simple mechanism by which a ubiquitylated protein can be incorporated into clathrin-coated vesicles and transported from the trans-Golgi network to endosomes (Figure 2).

Though these studies were initially performed with mammalian GGA proteins, ubiquitin-mediated diversion from the Golgi to endosomes has not so far been demonstrated in mammalian cells. In yeast, however, it is well established both that GGAs are required for specific transport to endosomes [9], and that ubiquitylation of, for example, the Gap1 amino acid permease is necessary for its entry into this pathway [10,11]. Yeast GGAs also bind ubiquitin, and construction of a mutant strain whose only GGA protein lacks the ubiquitin-binding domain demonstrates clearly that it is the ubiquitin–GGA interaction that causes diversion of Gap1 to the vacuole [6].

The subsequent incorporation of ubiquitylated membrane proteins into multivesicular bodies depends on interactions with several different peripheral membrane proteins with ubiquitin binding domains, notably Vps27/Hrs and Hse1/STAM — both of which have a ubiquitin interaction motif (UIM) — and Vps23/TSG101 [12–14]. These proteins have an interesting property that derives from the nature of the ubiquitylation process. Ubiquitin is first linked to a cysteine residue on a ubiquitin conjugating enzyme, before being transferred to the ultimate substrate with the aid of a ubiquitin ligase. Ubiquitin binding proteins can bind directly to the activated intermediate, and they often catalyse their own ubiquitylation [15]. This in turn may allow the formation of localised networks or patches in which the proteins bind each other, into which ubiquitylated cargo would be incorporated. The GGA proteins are also ubiquitylated [7], suggesting that the same mechanistic trick is used, not only throughout the endocytic pathway, but also on the trans-Golgi network.

Interestingly, human GGA3 is present not only on the trans-Golgi network but also on endosomes [5], as is clathrin. Furthermore, endosomal clathrin is associated with Hrs, and appears to be involved in gathering components that later help to form, or enter into, the invaginating membranes of multivesicular bodies [16,17]. Knockdown of GGA3 by RNA interference (RNAi) hinders this process, causing inflated endosomes to accumulate and blocking the transport of EGF receptor

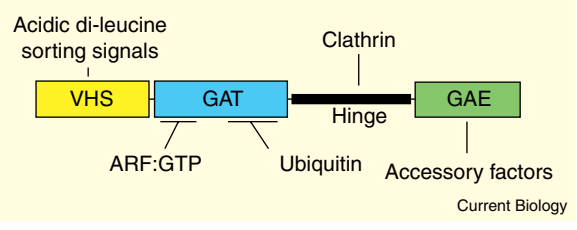

Figure 1. Domains and interactions of a typical GGA protein.

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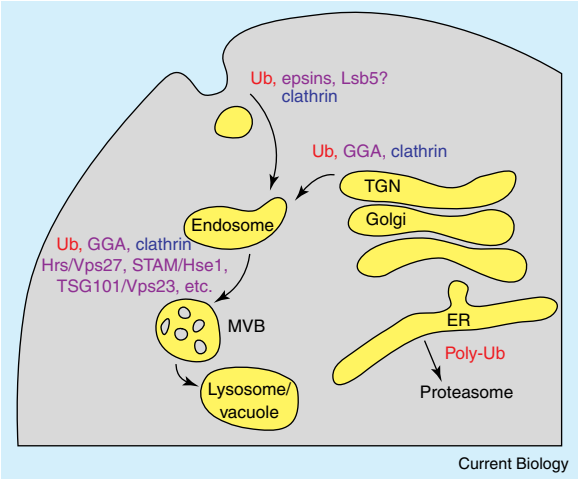

Figure 2. Pathways to destruction.

Illustrated are the steps for which ubiquitin (Ub) serves as a signal, and some of the other components involved. The purple components are (or in the case of Lsb5 may be) ubiquitin-binding proteins that are themselves ubiquitylated, and thus may both recognise substrates and form networks. Note that entry into the internal vesicles of multivesicular bodies probably requires sequential interactions of ubiquitylated substrates with the endosomal proteins listed, as well as others. MVB, multivesicular body; TGN, trans-Golgi network; ER, endoplasmic reticulum.

molecules to lysosomes [5]. Thus, GGAs and clathrin play an important role, not just in transporting material to endosomes, but also in its delivery into multivesicular bodies. Yeast GGAs also contribute to endosome maturation, a function that is, however, at least partly distinct from their ubiquitin binding activity [6]. A continuing role for GGAs in early endosomes may explain why GGA-dependent traffic from the yeast Golgi leads efficiently to late, rather than just early, endosomes [9].

The function of GAT domains may extend further. Ubiquitin binding was first demonstrated for the GAT domain of Tom1, a protein that is similar to the GGAs but lacks the GAE domain [18]. Tom1 binds another ubiquitin-binding protein, Tollip, but its precise function is unknown. The yeast protein most similar to Tom1 is Lsb5, which is known to interact with the endocytic machinery and the actin cytoskeleton, to have a cortical location, and to contribute to the endocytic pathway [19]. Yeast differs from animal cells in that clathrin is relatively unimportant for endocytosis, but monoubiquitination of plasma membrane proteins clearly provides an endocytic signal. Thus, Lsb5 may be involved in early steps in endocytosis, alongside the epsin-like proteins Ent1/2 and Ede1, which have UIM domains and are known to be involved in endocytosis [14].

Several generalisations can be drawn from these findings. First, the use of networks of ubiquitin-binding and ubiquitylated proteins to create sorting domains may be common to endosomes, the trans-Golgi network and perhaps the plasma membrane. Second, ubiquitylated membrane proteins (as with cytosolic proteins) seem inevitably destined for destruction — they can be gathered from Golgi, plasma membrane and endosomes and targeted into multivesicular bodies. Third, clathrin, GAT domain proteins and other ubiquitin-binding proteins contribute to this process at each location. Ubiquitylated proteins seem to be passed on from one binding protein to another as they pass inexorably towards multivesicular bodies. In contrast, recycling proteins such as the mannose-6-phosphate or transferrin receptor interact only with proteins early in the pathway, such as the GGAs or the AP-2 clathrin adaptor proteins on the plasma membrane, and thus can later escape and be retrieved.

These common themes easily explain evolutionary variation — additional ubiquitin- or clathrin-binding proteins may extend the range of pathways or substrates without fundamentally altering the underlying goal of lysosomal delivery. Conversely, it is likely that pathways that serve a different purpose use quite different sorting signals and recognition machinery — it would be hard to evolve a completely independent function for clathrin or ubiquitin, because it would be necessary simultaneously to prevent interactions with the existing machinery. It follows that, for example, endosome–Golgi retrieval is unlikely to involve clathrin or its associates, and indeed the sorting nexins are better candidates for this role [20].

This apparently simple view should not blind us to exceptions. It is unlikely, for example, that monoubiquitylated histones are substrates either for the proteasome or for multivesicular body sorting, and even for membrane proteins, rapid removal of ubiquitin may allow short-term functions that do not involve degradation. It is also easy to imagine mechanisms that would allow one coat protein to perform two roles. But what is becoming clear is that the principles of protein sorting are more general, and perhaps simpler, than they have sometimes appeared.

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